Affimers – Next Generation Affinity Tools

POS003

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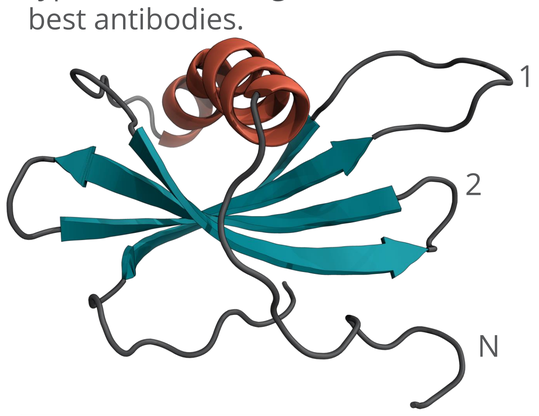
Introduction

The Affimer protein scaffold is a biologically inert, biophysically and biochemically stable scaffold capable of presenting a range of designed or random binding surfaces (defined by peptides inserted at loop 1, loop 2 and the amino terminus) for highly specific, high affinity interactions with a wide range of targets. There are two versions of the scaffold, one based on mammalian Stefin A (Stadler *et al*., 2011) and a second based on plant Cystatin A (Tiede *et al.,* 2014). The tertiary structure of both types are homologous. Affimers are designed to work in the same way as the very best antibodies.



Loop lengths can be varied to change the size or shape of the recognition surface. Our current libraries use versions of the scaffold where Loop 1 is 6 residues long and Loop 2 is 12 residues long or where both loops have an equal length of 9 residues.

Candidate binders are rapidly isolated using a three or four-round phage display process which takes approximately two weeks. Screens can be undertaken with or without competition, as required by the target type.

Stability

Both scaffold types demonstrate good thermal stability and resistance to high and low pH.

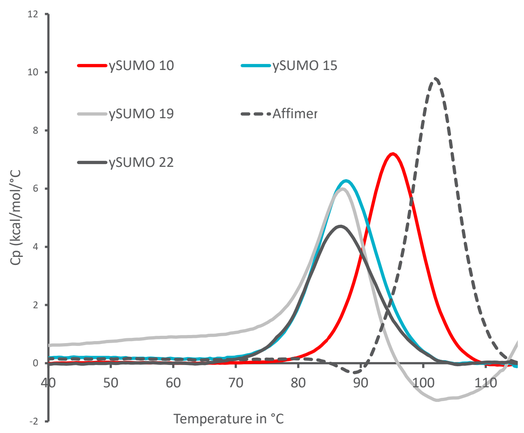


Figure 1. DSC analysis of the Affimer core scaffold and 4 variants that bind Yeast SUMO

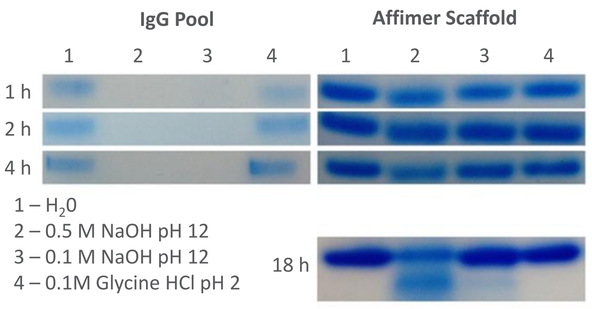
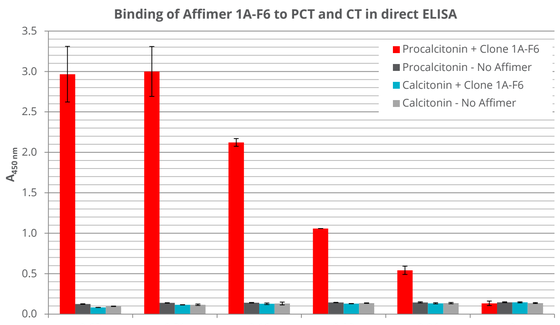
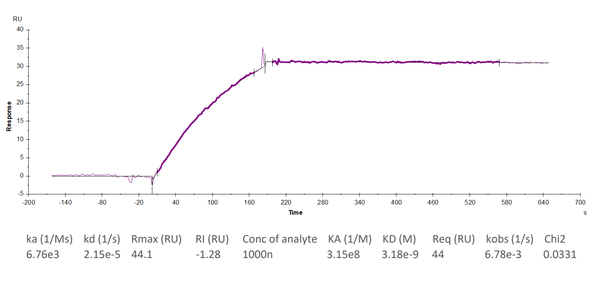


Figure 2. pH stability of the Affimer core scaffold compared to an IgG pool

ELISA & SPR

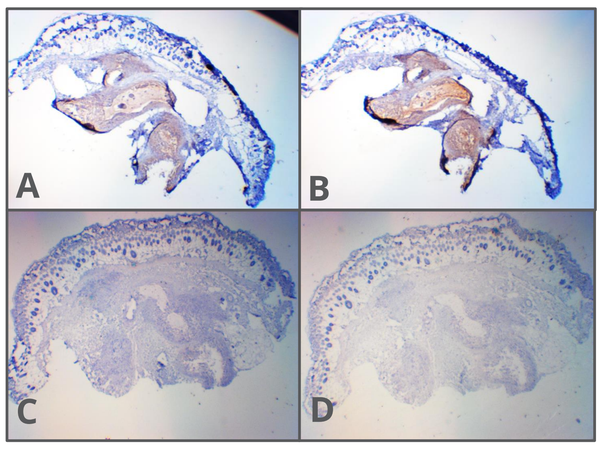
Binders were generated to Procalcitonin in 7 weeks. The lead binder has a K_d of ~3 nM and LOD of ~ 0.04 µg/mL in a non-signal amplified direct ELISA.





ka (1/Ms)	kd (1/s)	Rmax (RU)	RI (RU)	Conc of analyte	KA (1/M)	KD (M)	Req (RU)	kobs (1/s)	Chi2
6.76e3	2.15e-5	44.1	-1.28	1000n	3.15e8	3.18e-9	44	6.78e-3	0.0331

IHC



Immunohistochemistry using an Affimer to Tenascin C (TNC).

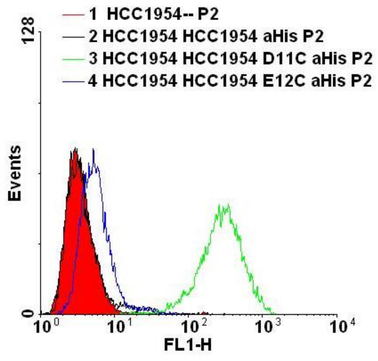
Panel A shows the staining pattern in U251 tumor tissues when using the anti-TNC affimer whilst panel B shows equivalent staining with an IHC grade anti-TNC antibody. Panels C & D are the no affimer and no antibody control samples, respectively.

References

Stadler *et al*. (2011) Structure-function studies of an engineered scaffold protein derived from Stefin A. II: Development and applications of the SQT variant. PEDS 24(9) 751-63.

Tiede *et al*. (2014) Adhiron: a stable and versatile peptide display scaffold for molecular recognition applications. PEDS 27(5) 145-155.

Flow Cytometry



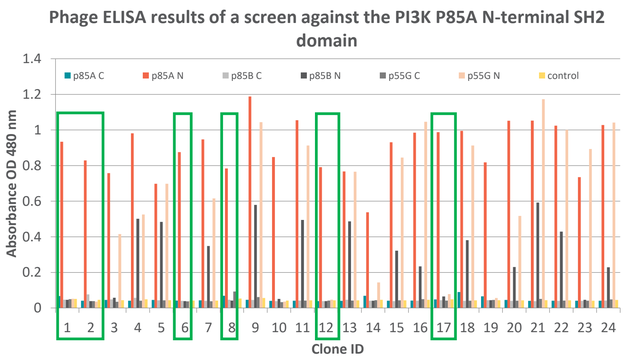
Binders raised against HER2 were tested for utility in flow cytometry against HCC1954, a human breast carcinoma-derived cell line expressing high levels of HER2 .

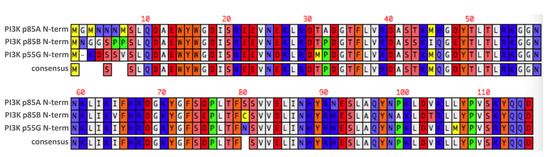
Affimer clone D11C showed separation of HCC1954 cells when incubated (using 4 µg/mL Affimer) with cells for 30 min on ice before incubation with an anti-pentaHis (AlexaFluor 488) antibody for a further 30 min on ice.

An Affimer from the same screen, E12C, did not function in this application.

Isoform Specificity

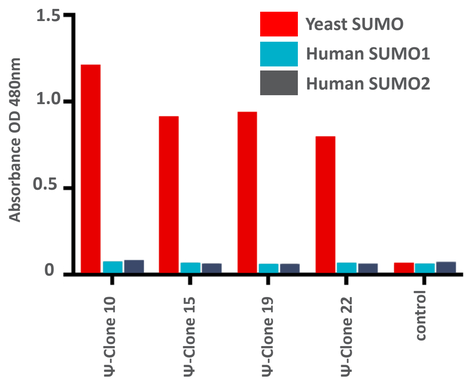
Binders were generated to the SH2 domains of PI3K subunits. The data shows several binders specific to the N-terminal SH2 domain of PI3K p85. The sequences of the N-terminal SH2 domains are shown.

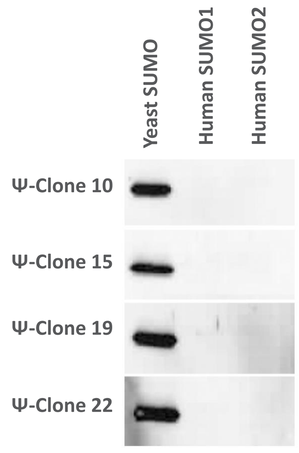


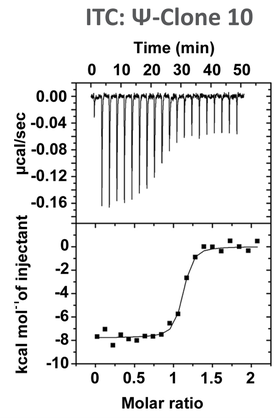


Species Specificity & Western Blotting

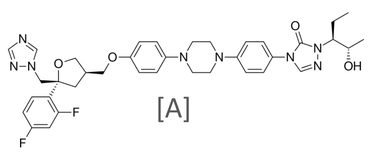
Binders were generated that were specific to yeast SUMO. Data shown are direct ELISA and western blot results for four candidates against yeast SUMO and human SUMO 1 & 2. ITC for one candidate shows a K_d of ~30 nM.

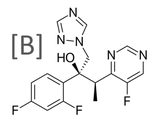




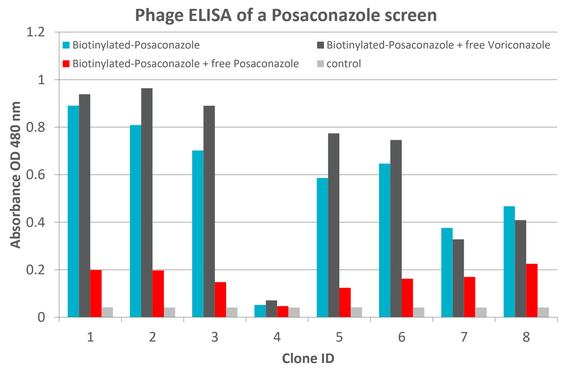


Small Molecule Binders





Positive Phage ELISA against a small-molecule, Posaconazole [A]. Positive candidates do not react with the linker or a closely related molecule, Voriconazole [B], in a competition ELISA.



Summary

Affimers are small, robust proteins and can be developed rapidly (7 weeks) against a range of target classes including those which are not tractable using animal-based methods of antibody generation.

The screening methodology used allows the engineering of specificity during the selection process. As this is a small protein with no modifications or disulphide bridges, there are none of the batch-to-batch variation issues that can plague antibodies. The Affimer scaffold should be easy to functionalise by both chemical modification and genetic fusion.

Affimers can be used in all typical antibody applications with the added benefit of being able to be used intracellularly to manipulate protein pathway. This suggests that, in the medium to long term, they would also be ideal tools for targeted live-cell imaging and drug target validation.

For further information regarding Affimer technology, please contact chris.miller@avacta.com or visit www.avacta.com